



# Green Student Lab

## *RiboNix: removal of rRNA from total RNA for sequencing in various plant species*

In the past decades the study of gene expression profiles has taken an enormous flight. The DNA microarray has been the standard tool to study transcriptomics for twenty years. With the advent of next-generation sequencing the past five years have shown a switch to RNA-seq as the preferred method to conduct gene expression studies. RNA-seq has considerable advantages over microarrays in terms of noise reduction, dynamic range and novel transcript detection. However, it does require different handling of RNA samples as the majority of total RNA consists of rRNA (>98%). To circumvent wasting sequence reads the rRNA has to be removed from the total RNA. Commercially available kits have shown little applicability in plant species and are relatively costly up to this point. This project aims to develop a general tool for rRNA removal (RiboNix) from different plant species consisting of a set of biotinylated DNA oligos which hybridize to cytoplasmic and chloroplast rRNA followed by extraction through streptavidin-coated magnetic beads. Tool development will comprise comparing rRNA from different plant species, designing and testing oligos, as well as optimization of the protocol conditions.

In addition, this approach will be compared to another method of mRNA enrichment which makes use of the poly-A tail present in most mRNAs in eukaryotic cells. We expect the later method to be unfavorable due to a potential loss of non-polyadenylated transcripts. We aim to identify novel non-polyadenylated RNAs to confirm these suspicions and strengthen the argument to use an approach based on the removal of rRNA from total RNA instead of a method based on extraction of mRNA.

Involved company:

MAD: Dutch Genomics Service & Support Provider

Research questions:

- Is it possible to develop a tool that can generally be applied in plants to remove rRNA from total RNA?
- Can we identify novel non-polyadenylated RNAs that justify the development of the RiboNix tool?

Techniques:

- BLAST
- Oligo design
- RNA extraction
- rRNA removal (RiboNix)
- polyadenylation
- micro-array